

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
21 October 2004 (21.10.2004)

PCT

(10) International Publication Number
WO 2004/090104 A3

- (51) International Patent Classification⁷: **C12Q 1/68** **Jlalu [CN/US]; 30 Irving Street, #21, Cambridge, MA 02138 (US).**
- (21) International Application Number: **PCT/US2004/010341** (74) Agents: **GAUDET, Stephen, J. et al.; Perkins Smith & Cohen LLP, One Beacon Street, 30th Floor, Boston, MA 02108-3106 (US).**
- (22) International Filing Date: **2 April 2004 (02.04.2004)**
- (25) Filing Language: **English** (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): **AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.**
- (26) Publication Language: **English** (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): **ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).**
- (30) Priority Data: **60/459,897** **2 April 2003 (02.04.2003)** **US**
- (71) Applicants (*for all designated States except US*): **PRESIDENT AND FELLOWS OF HARVARD COLLEGE [US/US]; Holyoke Center, Suite 727, 1350 Massachusetts Avenue, Cambridge, Massachusetts 02138-5701 (US). REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; Office of Technology Transfer, University of California, Office of the President, 1111 Franklin Street, 5th Floor, Oakland, California 94607-5200 (US).**
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **XIE, X., Sunney [US/US]; Harvard University, Office for Technology and Trademark Licensing, Holyoke Center, Suite 727, 1350 Massachusetts Avenue, Cambridge, MA 02138 (US). XIAO, Jie [US/US]; Harvard University, Office for Technology and Trademark Licensing, Holyoke Center, Suite 727, 1350 Massachusetts Avenue, Cambridge, MA 02138 (US). CAI, Long [US/US]; Harvard University, Office for Technology and Trademark Licensing, Holyoke Center, Suite 727, 1350 Massachusetts Avenue, Cambridge, MA 02138 (US). MARKSON, Joseph, Scott [US/US]; 6127 Cedar Wood Drive, Columbia, MD 21044 (US). YU, Ji [US/US]; Harvard University, Office for Technology and Trademark Licensing, Holyoke Center, Suite 727, 1350 Massachusetts Avenue, Cambridge, MA 02138 (US). YIN,**
- Published:**
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
- (88) Date of publication of the international search report: **3 March 2005**
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **DETECTING GENE EXPRESSION IN LIVE CELLS USING SHORT-LIVED REPORTERS WITH ENZYMATIC AMPLIFICATION**

(57) Abstract: Disclosed herein are compositions and methods for detecting and analyzing both transcriptional and translational events occurring in live cells. In particular, short-lived reporters with enzymatic amplification are described. These reporters have relatively short maturation time and a short cellular lifetime which can be exploited to detect transient events of gene expression in live cells.



WO 2004/090104 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/10341

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/68

US CL : 435/6, 325, 252.33

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 325, 252.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, BRS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WORLEY. C.K. Engineering in vivo instability of firefly luciferase and Escherichia coli beta-glucuronidase in higher plants using recognition elements from the ubiquitin pathway. Plant Molecular Biology. 1998, Vol. 37, pages 337-347, see entire document.	1,2,5-7,9,10,21, 30-32,37-40
X	DANTUMA. N.P. Short-lived green fluorescent proteins for quantifying ubiquitin/proteasome-dependent proteolysis in living cells. Nature Biotechnology. May 2000, Vol. 18, pages 538-543.	1-6,9-12,21, 30-32,37-40

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

08 November 2004 (08.11.2004)

Date of mailing of the international search report

27 DEC 2004

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703)305-3230

Authorized officer

James S. Ketter
Telephone No. (703) 308-0196

DEBORAH A. THOMAS
PARALEGAL SPECIALIST

GROUP 1360